Etiology

Pathogenicity of *Alternaria alternata* on Potato in Israel

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ABSTRACT

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A fungal pathogen responsible for brown spots on the underside of potato leaves in Israel was identified as *Alternaria alternata*. The fungus infected the leaf by direct penetration and via stomata. Young plants, at the 10–12 leaf stage, were less susceptible than adult plants; a differential susceptibility of the leaves was observed in which the middle leaves of the plant showed the highest disease incidence at any given growth stage. Susceptibility varied also according to the cultivar and the rate of sprinkler irrigation. Under field conditions, 28% foliar disease incidence significantly reduced tuber yield (18%). Fungicidal sprays with maneb, imazalil, or iprodione reduced the severity of disease significantly. The evidence indicated that *A. alternata* is a new pathogen of the potato crop in Israel.

Additional key words: Solanum tuberosum.

In recent years, brown spots that develop on the lower side of potato leaves were observed in potato fields in Israel (Fig. 1). Several nonbiological and bacterial agents were considered as possible causes but no conclusive results were obtained. In a preliminary study (8), the fungus *Alternaria alternata* (Fr.) Kreissler was consistently isolated from these spots. This fungus was not described as a pathogen of potato leaves, but was reported as a leaf pathogen in a number of other hosts such as pepper (2), tomato (4), bean (16), durum wheat (20), tobacco (12), persimmon (9), mango (10), and grey mangrove (1).

The objectives of this work were: to determine if *A. alternata* is the causal organism of the brown spot observed on potato leaves, to investigate environmental factors affecting disease development in

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the field, and to evaluate the relationship between foliar disease severity and tuber yield of potatoes.

MATERIALS AND METHODS

Potato plants (*Solanum tuberosum* L. 'Desiree,' 'Blanka,' 'Spunta,' 'Mirka,' 'Cardinal,' and 'Up-to-Date') were grown in a greenhouse at 20 C or in the field. The pathogen was isolated from characteristic spots on potato leaves (Fig. 1) after surface disinfesting 0.5-cm square leaf samples with 1% NaOCl for 1 min, washing them twice in sterilized distilled water, and incubating them at 25 C on potato-dextrose agar (PDA) for 4 days. Single-conidium cultures were used in all inoculations. Cultures were maintained on PDA at 25 C, and no changes in pathogenicity were observed throughout the experiments.

Inoculum was prepared in two ways: (i) The fungus was grown on S-medium (17) to induce the production of abundant aerial conidial chains. Spores were harvested by adding small amounts of water and rubbing gently with a bent glass rod. (ii) Slices (0.5–1.0 cm square) of 1-wk-old fungus culture grown on PDA at 25 C were shaken in sterile distilled water, and the suspension was filtered through cheesecloth to remove the hyphae and PDA remnants. The number of spores in the suspension was determined with a hemacytometer, and the concentrations were generally adjusted to 1.5×10^4 conidia per milliliter. Inoculation was done on freshly detached potato leaflets as well as whole plants. The leaflets were disinfested with NaOCl as described before, sprayed with 2 ml of spore suspension while over filter paper, and incubated on 1% water agar plates. Whole plants were sprayed with ~30 ml of spore suspension per plant. The plants were covered with polyethylene bags to maintain high humidity and incubated at 20 C for 2–3 days. Light microscope observations were made by clearing the leaf samples according to a method described by Shipton and Brown (18), or by fixing with 2% gluteraldehyde, dehydrating, and embedding in Spurr's resin as described by Luft (7). Samples of the cleared tissue and sections of the embedded tissue were mounted in immersion oil and examined with a phase-contrast microscope.

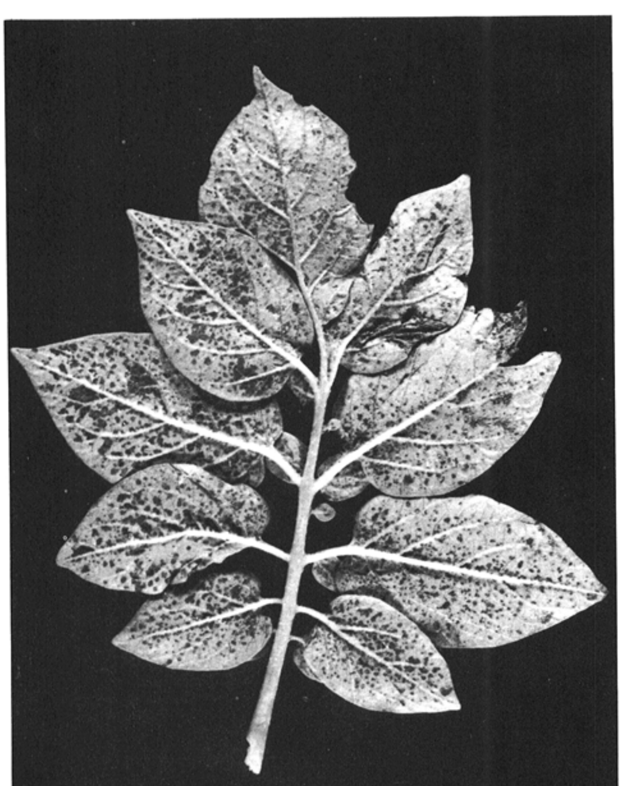

Fig. 1. Symptoms of the brown spot disease caused by natural infection of *Alternaria alternata* on leaf of potato cultivar Desiree observed from its lower side.

Development of the disease in commercial fields was followed by random sampling of eight plants per 0.5 ha of potato field. Estimates of the percentage of infection per leaf was made once every 2 wk by comparing the diseased leaves with a standard scale of leaves with different calculated percentages of covered area. Percentage of infection per plant is the average value of infected areas of the leaves present.

Field experiments to test cultivar susceptibility were conducted at the farm of the Faculty of Agriculture at Rehovot. The experimental design was a completely randomized block with seven replications, each composed of two 5-m-long rows. Plants were artificially inoculated at the 10- to 12-leaf stage by spraying with 4 L of spore suspension (1.5×10^4 conidia per milliliter) per 0.8 ha. Estimation of the disease level was done by evaluating the diseased area of all the leaves present on three plants in each replication.

The effect of physiological age of the plant on the severity of the disease was tested by planting potato seed tubers, cultivar Desiree, five times, once every 3–4 days, according to a completely randomized block design, with seven replications of two 5-m-long rows. The plants were inoculated when the oldest plants had 10–12 leaves, by spraying 5 L of spore suspension (1.5×10^4 conidia per milliliter) per 0.1 ha. Physiological age is expressed as the number of days from seeding.

The effect of different rates of sprinkler irrigation on disease level was tested on cultivars Desiree and Up-to-Date, according to a method described by Hanks et al (5). The experiments were designed in completely randomized blocks with three replications. Each replicate consisted of two rows of plants 6 m long, 3 m of which was planted with cultivar Desiree and the other 3 m with cultivar Up-to-Date. The different amounts of irrigation were obtained by planting the potato plants parallel to, and at different distances from, the sprinkler lines.

The effect of disease development on yield of tubers under field conditions was tested with the help of fungicide applications in a randomized block experiment with five replicates, 9×34 m each. Beginning at the 10- to 12-leaf stage, plants were sprayed with the fungicides maneb (80% W.P., Agan Chemicals, Ashdod, Israel) 2.5 kg/ha once a week after every irrigation, iprodione (50% W.P., Rhone-Poulenc, France) 1.0 kg/ha, before or after every irrigation, and imazalil (20 E.C., Janssen Pharmaceutica, Belgium) 0.5 L/ha, before every second irrigation. The crop was irrigated once a week. Disease development was estimated once every 2 wk. Potato yield was weighed after manually harvesting the tubers with a garden fork from two adjacent rows, 5 m length, in the middle of the replicate.

RESULTS

Determination of the causal organism. Conidia isolated on PDA medium from diseased potato leaves were obclavate and borne in long chains. The spores had one-to-seven transverse septa, one-to-two longitudinal septa, and were $26.7–28.0 \times 10.8$ μm in size (Table 1). This fungus was identified as *Alternaria alternata* (Fr.) Keissler by its similarity to descriptions published by Simmons (19) and Grogan et al (4) and was the only organism isolated from the brown spots. When detached leaves or whole plants were inoculated with suspensions of conidia of this fungus, disease symptoms developed after 3–5 days. Symptoms consisted of brown spots, a few millimeters in size, dispersed all over the leaf surfaces. Reisolation from the infected leaves indicated that *A. alternata* was the causal agent of leaf spots on potato. Different concentrations of spores, 10^3, 10^4, and 10^5 per milliliter, resulted in respective disease severities of 0.4, 0.5, and 0.86% infected leaf area, respectively, which also represent the relationship between the amount of inoculum and disease severity.

Penetration and colonization. *A. alternata* infected the leaf both by direct penetration through the epidermal cells (Fig. 2), and through stomata. Further development was inter- and intra-cellular, with necrotic cells noted surrounding the penetration site.

Plant and cultivar susceptibility to attack by *Alternaria*. Evaluation of disease severity in commercial potato fields of cultivars Up-to-Date, Cardinal, Spunta, Desiree, and Blanka, indicated that at the end of the season the cultivars Blanka, Desiree, and Spunta showed the greatest susceptibility. Cardinal was intermediate and Up-to-Date exhibited least susceptibility (Fig. 3A). Similar relations between the cultivars were observed the following season in commercial fields. The middle leaves of the plant were the most heavily infected part of the plant (Fig. 3B). This greater susceptibility (ie, higher percentage of area infected) of the middle leaves of the plant was observed in young (15 leaves) plants as well as in older plants.

Artificial inoculations of the cultivars in an experimental field also showed the differential susceptibility among cultivars reported above in the commercial field plots. Cultivars Desiree and Blanka showed high susceptibility, Spunta intermediate susceptibility, and Up-to-Date the least susceptibility (Fig. 4).

A significant increase in percent of diseased area was observed on adult plants. At the ages of 58, 63, 67, 70, and 74 days, the values of diseased area were 12.3, 21.5, 37.4, 39.3, and 47.2%, respectively. At each age, disease severity was the highest at the mid-height of the plant (Fig. 5).

Effect of overhead irrigation on disease development. The effect of irrigation regime on the development of brown spots on potato was tested in field experiments under conditions of natural infection. At the end of the growing season the highest severity of the disease, 10%, was obtained under large rates of overhead irrigation (56 mm/wk). The decrease in the irrigation rates to 44 and 34 mm/wk resulted in a decrease in the disease development to 8.0 and 4.5% of diseased area, respectively, on the plants. The differential pattern in which the middle of the plant suffers the highest disease severity was maintained in all irrigation regimes (Fig. 6). Disease development on cultivar Up-to-Date was very low, even under large rates of overhead irrigation.

Effect of disease area on yield. The application of maneb, iprodione, and imazalil as protectant treatments, starting when the plants had 12 leaves, resulted in a significant decrease in disease development during the growing season (Fig. 7).

A significant difference was observed between tuber weight from the control, 53.7 tonnes/ha, and the other treated plots: with maneb, 68.4 tonnes/ha; iprodione before irrigation, 66.2 tonnes/ha, iprodione after irrigation, 66.4 tonnes/ha; and imazalil once in 2 wk, 65.3 tonnes/ha, indicating that the disease caused by *A. alternata* had a significant effect on yield reduction. No other diseases were present in the experimental plot during the whole growing season.

Losses in potato yields resulted from the losses of green foliage. Multiple regression analysis gave best fit to the following values of disease severity on equation:

$$Y = 69.4 + 0.22X_1 - 0.188X_2 - 0.44X_3$$

in which X_1, X_2, and X_3 are the three dates of disease evaluation (58, 72 and 86 days after emergence, Fig. 7), considered as the independent variables, while the yield (Y) in each treatment was the dependent variable. The independent variables were compared to the dependent variable in each of the five replications of the treatments, so that 25 different disease severities and yield values were used for the equation. The equation is significant at 0.05%; indicating a significant effect of the disease level on the yield losses. In the best fitted model, the effect of the disease was expressed at the second and third dates of evaluation, but not at the first date. At the second date every 1% of diseased area reduced the yield by 0.188 tonne/ha and at the third date every 1% of diseased area reduced the yield by 0.44 tonne/ha. These reductions relative to the best yield obtained are equivalent to 0.28 and 0.65% decrease in yield per 1% increase of disease incidence at the last two dates, respectively.

DISCUSSION

Completion of Koch's postulates, and the increase in disease incidence resulting from increased inoculum concentration, support the conclusion that the brown spots of potato leaves are caused by *A. alternata*. We have not found any reports of a similar disease in potato with differential cultivar susceptibility caused by

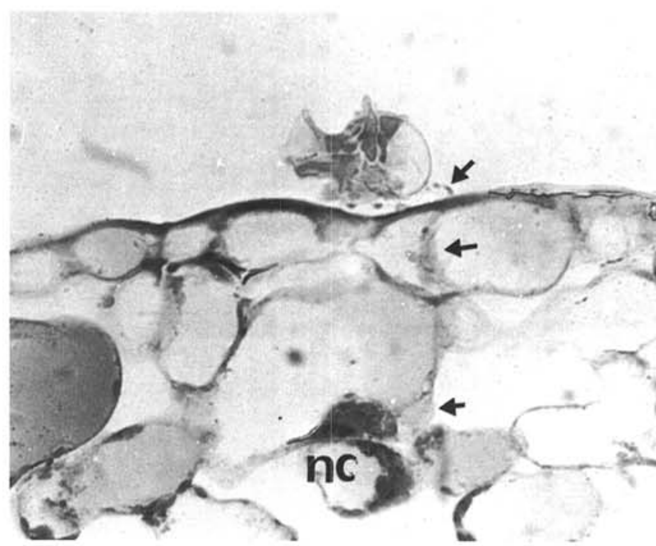

Fig. 2. Direct penetration of leaves of potato cultivar Spunta by *Alternaria alternata*. Observe the developing hyphae (arrows) and the presence of necrotic cells (nc) (×800).

TABLE 1. Morphological comparisons of conidia of *Alternaria* from potato leaves, with published descriptions of *Alternaria alternata* and *A. tenuis*

		A. alternata			*A. alternata* f. sp. *lycopersici* (14)	
		Potato leaves[a]		Neotype specimen from unidentified pithy stem (19)	Tomato stem from field	*A. tenuis* (3)
Criteria		cv. Desiree	cv. Draga			Potato leaves
Spore body						
length (µm)	Range	13.2–46.2	14.8–46.2	18–47	18–50	7–59
	Mean	28.0	26.7	30.9	32.2	24.1
	S.D.	6.0	4.9	···	2.8	8.4
width (µm)	Range	6.6–13.2	6.6–14.9	6.6–16.5	7–18	5–15
	Mean	10.9	10.9	12.6	12.4	10.4
	S.D.	1.9	2.3	···	2.8	1.7
beak length (µm)	Range	1.7–6.6	1.6–8.2	up to 25	2.2	2–14
	Mean	3.6	3.6	···	6.8	6.0
	S.D.	2.0	2.0	···	6.0	4.3
No. of septa		1–6	1–6	3–8	1–5	···
Beaked spores (%)		88.7	88.5	···	72	54

[a] Three hundred conidia developed in cultures on PDA medium were included in the observation.

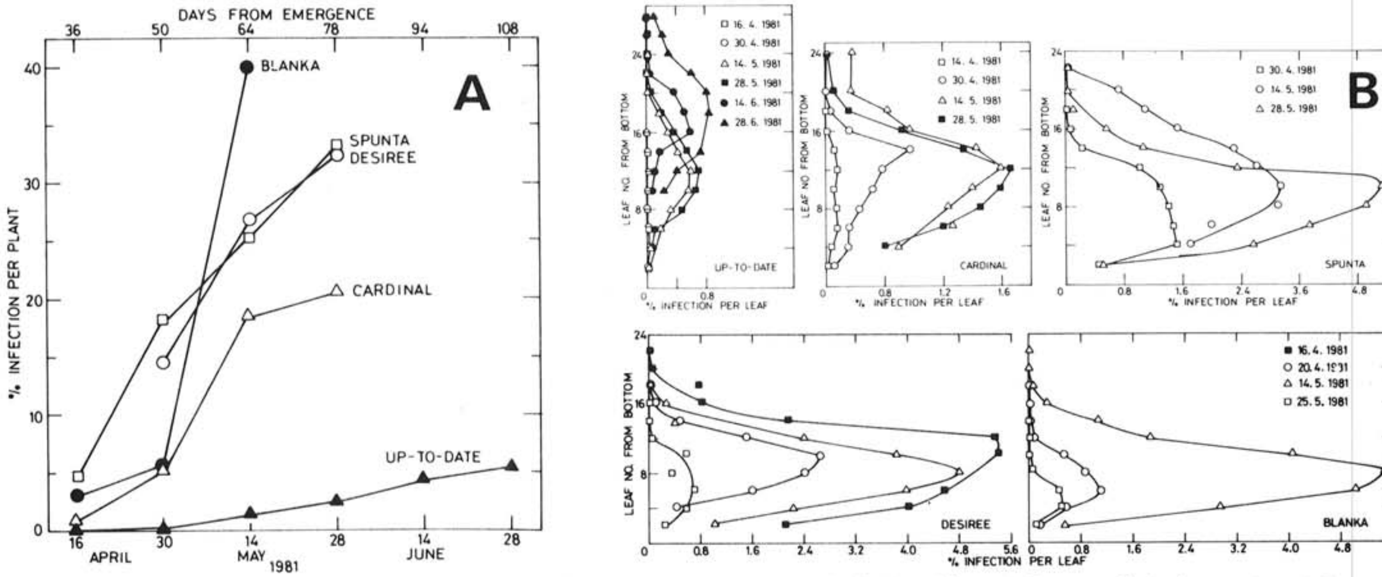

Fig. 3. Disease development caused by *Alternaria alternata* in different cultivars on commercial field conditions. **A,** Percent of infection per plant. **B,** Percent of infection per leaf. Percentage of infected area was obtained by comparison to a scale with different percentages of diseased area. Disease of every odd leaf of the figure represents the average of the disease incidences of that leaf and the opposite leaf beneath. Percentage of infection per plant is the average of diseased area of the leaves present. Emergence was observed on 11 March 1981; the dates in the figure represent when the disease was evaluated.

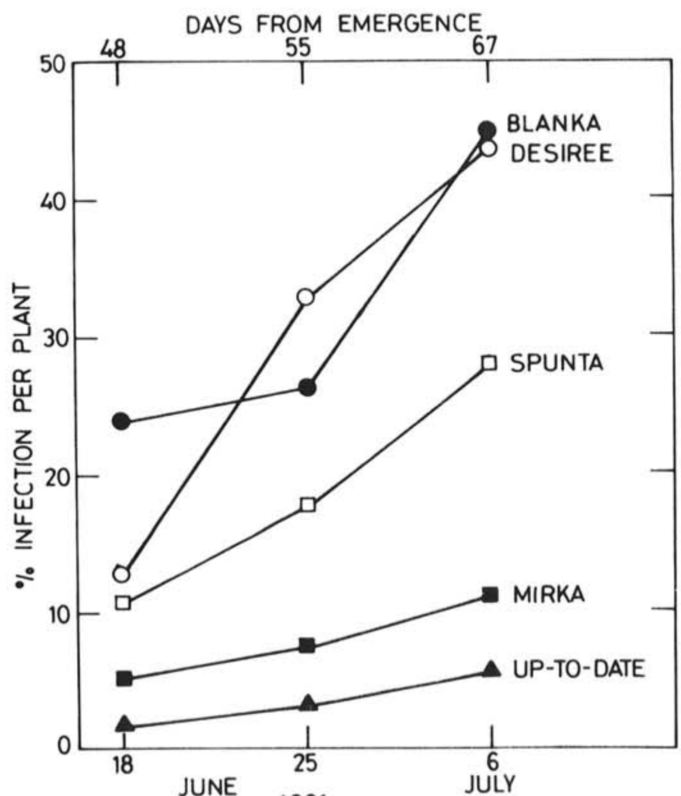

Fig. 4. Disease development caused by *Alternaria alternata* on plants of different potato cultivars. Inoculation was done in an experimental field as described in Materials and Methods when potato plants developed 10–12 leaves. The dates in the figure represent the dates when the disease was evaluated. The infected area represents the average percentage of infection of the leaves present. Percentage of infected area was obtained by comparison to a scale with different percentages of diseased area.

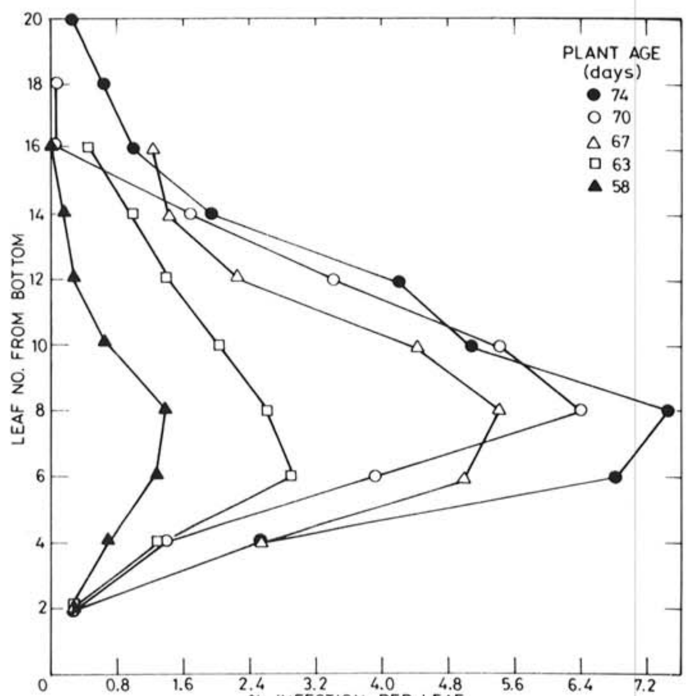

Fig. 5. Effect of physiological age of potato plants cultivar Desiree on the percentage of infected area caused by *Alternaria alternata*. Tubers were planted five times every 3–4 days and inoculation was conducted when the first planted potato plants developed 10–12 leaves; evaluation was done 4 wk later. Disease of every odd leaf of the figure represents the sum of disease incidence of that leaf and the opposite leaf beneath.

Alternaria, except that reported by Giha (3), who described broad necrotic spots. However, no mention was made of a brown spotting that is the major symptom of the disease.

A. alternata was found to penetrate the leaf either directly or through stomata, followed by further developments in the tissue causing a necrosis in the epidermal cells. This result is in agreement with the ability of germ tubes of *A. alternata* to penetrate persimmon fruit directly; apricots, through stomata; mango fruits, via lenticels (9,10); and tobacco leaves, either directly or through stomata (23).

As described before (21), in tobacco, the fungus was able to infect young and healthy potato plants; this is in disagreement with a report (16) that on bean the fungus causes disease on plants with low vigor, poor nutrition, or advancing senescence. Physiological age of the plant or plant organ, however, had a significant effect on disease severity. Four weeks after inoculation, adult plants showed four times more diseased area than young plants. Similarly, Saad and Hagedorn (16) reported that 3-wk-old bean plants were less susceptible than 4- to 6-wk-old plants. Tomato plants also showed increased infection with increased age, and older leaves were more susceptible than younger ones (14). Stavely and Slana (21,22) reported that tobacco leaf infection by *A. alternata* was governed by age and the leaves were infected by the fungus at any age, but that lesions on young leaves were very small in relation to the conspicuous necrotic spots produced in older leaves. Our results indicate that differences in disease severity were found between leaves on the same plant, along with differences resulting from the physiological age of the plant. The most susceptible leaves were those between the bottom third and the middle of the plants. Similar results were found by Koren (6) in tomatoes infected by *Leveillula taurica* (Lev.) Arn, in which the leaves in the middle of the plant were the most susceptible and showed the highest disease incidence, possibly as a result of the physiological state of the individual leaves. Suceptibility to *A. alternata* in potato leaves also varied according to the cultivar, and cultivar susceptibility increased in this order: Up-to-Date, Cardinal, Mirka, Spunta, Blanka, and Desiree.

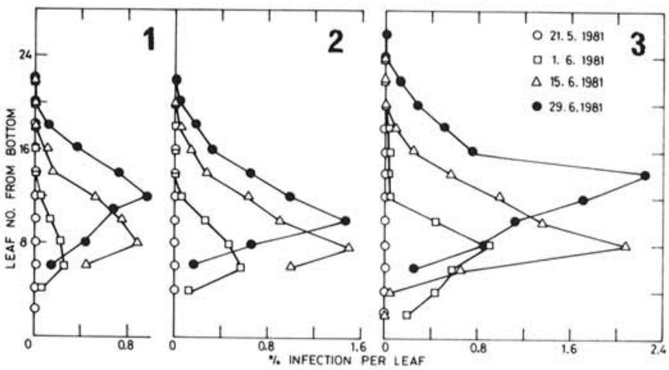

Fig. 6. Effect of different rates of overhead irrigation on the natural development of infected area by *Alternaria alternata* on potato plants of cultivar Desiree during spring 1981. Disease of every odd leaf of the figure represents the sum of disease incidence of that leaf and the opposite leaf beneath. The numbers 1, 2, and 3 represent irrigation rates of 34, 44, and 56 mm/wk, respectively. Dates in the figure represent the dates when disease evaluations were made. Emergence was recorded on 20 March 1981.

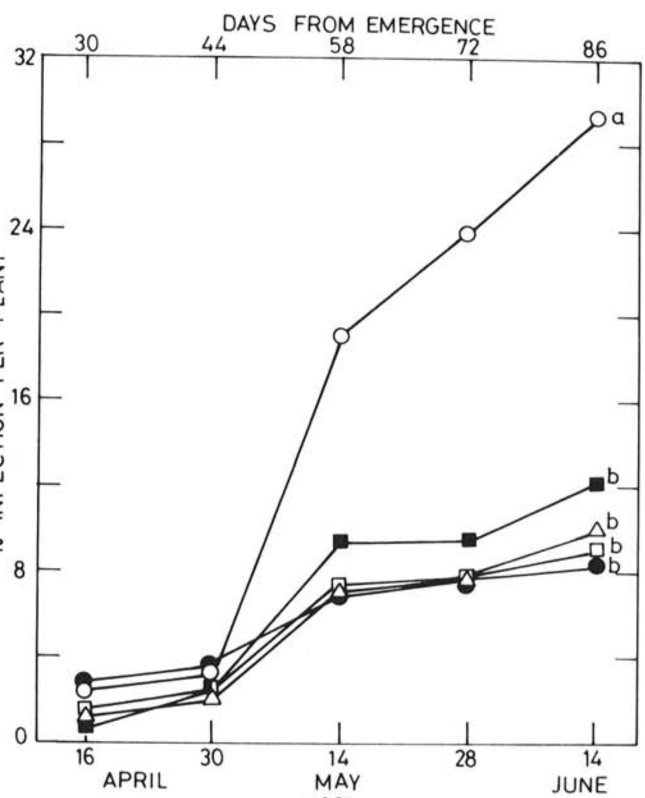

Fig. 7. Effect of fungicidal spray on the incidence of natural infection by *Alternaria alternata* on potato plants of cultivar Desiree. Symbols: O——O, untreated control; ●——●, iprodione, 1 kg/ha before every second irrigation; Δ——Δ, iprodione, 1 kg/ha before each irrigation; ■——■, maneb, 2.5 kg/ha after each irrigation; and □——□, imazalil, 0.5 L/ha before every second irrigation. Irrigation began immediately after seeding, and the field was irrigated once a week. Fungicide treatment started when the plants developed 10–12 leaves. Different letters indicate significance between treatments obtained by Duncan's multiple range test, $P = 0.05\%$.

The results presented here confirm previous reports (13) of infection by *Alternaria* species in which high humidity, leaf wetness, and suitable temperature are important factors. Long dew periods increased the infection rate of *A. alternata* on tobacco (22) and *A. solani* on potato crops (15). When combined with appropriate temperatures, the fungus caused severe epidemics (8,11,13,16). In our experiments, disease development also was increased by increasing the irrigation rate, possibly as a result of the humid conditions and the long duration of free water on the leaf surface. These humid conditions represent the normal irrigation rates used for commercial potato production in Israel.

The increased development of the disease in older plants is similar to findings with *A. solani* which showed that inoculum buildup is slow and the disease does not become destructive until late in the season (14). The causes for this slow buildup of inoculum concentration might be explained by the low susceptibility to infection of young and middle-aged plants, the generally small number of spores produced by *Alternaria* in living plant tissues, and production of spores of *Alternaria* mostly on dying and dead leaves.

The relatively new appearance of *A. alternata* on potato crops might be explained by on the introduction of new genetic background in the recently introduced cultivars or natural selection of more aggressive isolates of the fungus enhancing the incidence of *Alternaria*.

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